

X-12553  
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Erythropoietic Compounds

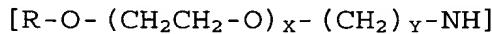
33. (new) A protein selected from the group consisting of:
- a) NGE[5E];
  - b) MR-NGE;
  - c) MR-NGE-88E;
  - d) MR-NGE-88K;
  - e) MR-NGE-88P;
  - f) MR-NGE-88S;
  - g) MR-NGE[4E];
  - h) MR-NGE[5E];
  - i) MR-NGE[5K];
  - j) MR-NGE[W5E];
  - k) MR-NGE[W5K];
  - l) NGE[5E]-166Δ;
  - m) MR-NGE-166Δ;
  - n) MR-NGE-88E-166Δ;
  - o) MR-NGE-88K-166Δ;
  - p) MR-NGE-88P-166Δ;
  - q) MR-NGE-88S-166Δ;
  - r) MR-NGE[4E]-166Δ;
  - s) MR-NGE[5E]-166Δ;
  - t) MR-NGE[5K]-166Δ;
  - u) MR-NGE[W5E]-166Δ; and
  - v) MR-NGE[W5K]-166Δ.

34. (new) The protein of Claim 33, wherein the protein is MR-NGE-166Δ.

35. (new) The protein of Claim 33, wherein the protein is MR-NGE[W5K]-166Δ.

36. (new) The protein of Claim 33, wherein the protein is MR-NGE[W5E]-166Δ.

37. (new) An erythropoietic compound having a protein portion and a polymer portion, wherein the protein portion is selected from the group consisting of: non-glycosylated human erythropoietin and non-glycosylated erythropoietin analogs and wherein the polymer portion consists of 1 to 5 polymer chains of the formula:



wherein R is H or C<sub>1</sub> to C<sub>4</sub> alkyl, X is a number from about 70 to about 1200, and Y is a number from 1 to 4; and the polymer chain is covalently bonded to the protein portion by a secondary amine bond.

38. (new) The erythropoietic compound of Claim 37 wherein X is a number from about 225 to about 1200.

39. (new) The erythropoietic compound of Claim 38 wherein X is a number from about 340 to about 1200.

40. (new) The erythropoietic compound of Claim 39 wherein X is a number from about 450 to about 1200.

41. (new) The erythropoietic compound of Claim 40 wherein X is a number from about 450 to about 700.

42. (new) The erythropoietic compound of Claim 37 wherein the protein portion is a non-glycosylated erythropoietin analog and the polymer portion is bound to the protein portion at the N-terminus of the protein.

43. (new) The erythropoietic compound of Claim 37 wherein the protein portion is a protein of Claim 33.

44. (new) A protein which is the product of the expression in a host cell of an exogenous DNA sequence comprising a DNA sequence encoding at least one of the proteins of Claim 33.

45. (new) The erythropoietic compound of Claim 37 made by a process comprising the steps of:

- a) adding a polyethylene glycol-aldehyde polymer to a solution of non-glycosylated erythropoietic protein under conditions that permit the formation of an imine bond between an amino group of the protein and the aldehyde group of the polymer; and
- b) adding a reducing agent to reduce the imine bond to a secondary amine bond.

46. (new) An isolated nucleic acid sequence, comprising a polynucleotide encoding a protein of Claim 33.

47. (new) A vector comprising a nucleic acid sequence according to Claim 46.

48. (new) A host cell comprising the vector of Claim 47.

49. (new) A host cell expressing a protein of Claim 33.

50. (new) The host cell of Claim 49 wherein said host cell is *E. coli*.

51. (new) A transgenic or chimeric non-human animal, comprising at least one host cell according to Claim 49.

52. (new) A process for producing a protein comprising the steps of transcribing and translating the isolated nucleic acid of Claim 46 under conditions that the protein is expressed in detectable amounts.

53. (new) A process for preparing polymer-derivatized, non-glycosylated erythropoietic compounds, comprising the steps of:

- a) adding a polyethylene glycol-aldehyde polymer to a solution of non-glycosylated erythropoietic protein under conditions that permit the formation of an imine bond between an amino group of the protein and the aldehyde group of the polymer; and
- b) adding a reducing agent to reduce the imine bond to a secondary amine bond.

54. (new) The process of Claim 53, wherein the aldehyde polymer and the protein in the solution are present at a ratio of 0.08 to 24 on a mole polymer per mole protein basis.

55. (new) The process of Claim 54, wherein the ratio of aldehyde polymer to protein is 1 to 10.

56. (new) The process of Claim 53, wherein the solution is buffered and the added reducing agent is selected from the group consisting of sodium cyanoborohydride and sodium borohydride.

57. (new) The process of Claim 56, wherein the solution is borate-phosphate buffered with a pH between 7 and 9 and said reducing agent is sodium cyanoborohydride.

58. (new) A method for increasing the hematocrit levels in a mammal comprising the administration of a therapeutically effective amount of an erythropoietic compound of Claim 37.

59. (new) A pharmaceutical formulation adapted for the treatment of patients with insufficient hematocrit levels comprising an erythropoietic compound of Claim 37.